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# High-level production of human parathyroid hormone in *Bombyx mori* larvae and BmN cells using recombinant baculovirus

(PTH; cDNA; silkworm; cells; osteoblast function tests)

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SUMMARY

A full-length cDNA encoding human parathyroid hormone (hPTH) containing the prepro region was cloned into *Bombyx mori* baculovirus under the control of the polyhedrin promoter and polyadenylation sequences. After transfection and generation of the recombinant baculovirus, hPTH production was examined in silkworm larvae and BmN cell cultures. The larvae synthesized and efficiently secreted the correctly processed and authentic hPTH (9.4 kDa) with no sign of internal degradation. In BmN cells, the major secreted form was the correctly sized protein, but small amounts of degraded hPTH could also be detected in the medium by immunoblotting. Unlike the situation in larvae, prepro-hPTH could also be demonstrated intracellularly in BmN cells. The concentration of hPTH in the larval hemolymph was about 70 mg/l, as compared to approx. 55 μg/l in the medium per $7.5 \times 10^6$ cells. Recombinant hPTH (re-hPTH) from the hemolymph was purified by reverse-phase HPLC and subjected to chemical and biological analyses. The authenticity of the purified re-hPTH was confirmed by N-terminal sequencing, amino acid composition and a mass of 9425 Da, close to the theoretical value. The hormone showed high-affinity receptor binding and full biological potency in increasing cellular cAMP.

## INTRODUCTION

Human parathyroid hormone (hPTH) is synthesized in the parathyroid glands as a prepro-hormone consisting of 115 aa. During processing, the pre and pro parts of the hormone are sequentially cleaved off resulting in the formation of the mature 84-aa hormone (Cohn and MacGregor, 1981). hPTH (1–84) is secreted in response to a lowering of serum $Ca^{2+}$ ions, and its physiological function is to elevate serum $Ca^{2+}$ and to maintain the calcium and phosphate homoeostasis (Potts et al., 1982; Reeve et al., 1980). Prolonged and intermittent administration of low to medium doses of biologically active hPTH fragment has been shown to vigorously stimulate bone formation in animals and patients with osteoporosis (Reeve et al., 1980; 1991; Bradbeer et al., 1992).

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Abbreviations: *A*, absorbance (1 cm); aa, amino acid(s); ACN, acetonitrile; *B.*, *Bombyx*; *Bm*, *B. mori*; BmN, *Bm* ovarian cell line; BmNPV, *Bm* nuclear polyhedrosis virus; bp, base pair(s); Bv, baculovirus; cAMP, cyclic AMP; cDNA, DNA complementary to RNA; cpm, count(s) per minute; DEPC, diethyl pyrocarbonate; HPLC, high-performance liquid chromatography; hPTH, human PTH; *hPTH*, gene (DNA) encoding hPTH; kb, kilobase(s) or 1000 bp; MS, mass spectrometry; LLC-$PK_1$, porcine renal epithelial cell line; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PAGE, polyacrylamide-gel electrophoresis; PBS, phosphate-buffered saline; pfu, plaque-forming unit(s); PPG, polypropylene glycol(s); PTH, parathyroid hormone; PTHrP, PTH-related protein; re-, recombinant; *S.*, *Saccharomyces*; SDS, sodium dodecyl sulfate; TFA, trifluoro acetic acid; *UTR*, untranslated region(s); wt, wild type.

Considering the potential pharmaceutical importance of the hormone in treatment of bone metabolic disorders, attempts have been made to produce the hormone employing different expression systems such as *Escherichia coli*, *Saccharomyces cerevisiae* and mammalian cells (Rabbani et al., 1988; Høgset et al., 1990; Gabrielsen et al., 1990; Rokkones et al., 1994). The existence of internal protease sensitive domains has made hPTH susceptible to degradation and inactivation (Høgset et al., 1990; Gabrielsen et al., 1990). Thus, an optimalized system for expression of h*PTH* demands a correct processing without aberrant cleavage so that a high production efficiency can be obtained.

In this paper we compare the expression of h*PTH* in BmN cells and *Bombyx mori* (*Bm*) larvae using the *Bm* baculovirus with the polyhedrin promoter and regulatory sequences (Maeda, 1989a,b). The results demonstrate that the cells and the larvae are fully able to recognize the human signal and pro-part of hPTH.

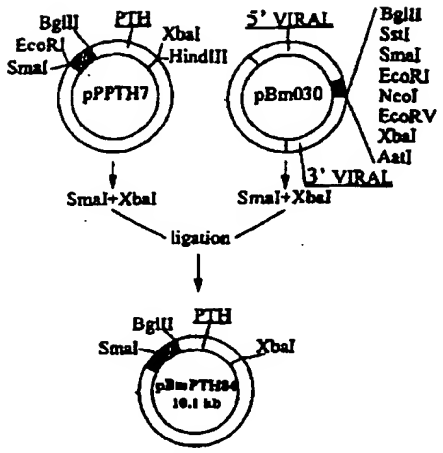


Fig. 1. Construction of baculovirus vector pBmPTH84. The entire h*PTH* cDNA signal and coding sequences were excised from the plasmid pPPTH7 by *SmaI* + *XbaI* digestion and ligated between the *SmaI* and *XbaI* sites of the vector pBm030 (Maeda, 1989b). In the resulting re-vector pBmPTH84 lacking the polyhedrin-encoding gene, the h*PTH* coding sequence is under the control of the polyhedrin promoter, transcriptional termination and polyadenylation sequences, but employing hPTH signal sequence. Methods: Plasmid DNA manipulations were performed essentially as described by Sambrook et al. (1989). A h*PTH* cDNA 413-bp fragment from plasmid pPPTH7 was ligated into vector pBm030, after cleavage with *SmaI* + *XbaI*, and transformed into the *E. coli* DH5α. The clones that contained the h*PTH* cDNA insert were identified and the re-vector named pBmPTH84. The junctions of h*PTH* cDNA and the transfer vector were confirmed by sequencing. Restriction enzymes and other DNA metabolizing enzymes were obtained from New England Biolabs. A anti-rabbit-[$^{125}$I]IgG was from Amersham. Synthetic hPTH (1–84) from Bachem was used as standard. All the other chemicals used were from Sigma.

## RESULTS AND DISCUSSION

### (a) Construction of recombinant virus for h*PTH* expression

Strategies followed for the cloning of full length h*PTH* cDNA (from pPPTH7) including its prepro part into the vector pBm030 is shown in the Fig. 1 and explained in legend. The re-vector pBmPTH84 harbours the full-length h*PTH* cDNA, including the human signal(pre) sequence and its pro part and is controlled by virus regulatory elements. Cotransfection of BmN cells in culture with the plasmid pBmPTH84 DNA and wt viral DNA (BmNPV) resulted in the formation of polyhedrin-negative re-plaques. Upstream and downstream from the cloned h*PTH* cDNA, about 3 kb viral flanking sequences are present, and during cotransfection, these flanking regions will facilitate homologous recombination so that the polyhedrin gene of the wt virus is replaced with the h*PTH* cDNA. After identification and isolation of re-viral plaques they were purified as described in Methods in the legend to Fig. 2. The re-virus were screened and those giving highest expression of h*PTH* were chosen for further experiments.

### (b) Production and secretion of hPTH into larval hemolymph

Hemolymph samples from larvae infected with re-virus and collected after 24, 48 and 72 h, showed a time-dependent increase in two peptides (9.4 kDa and 14.3 kDa) which immunoreacted with hPTH antiserum, while hemolymph from wt virus-infected larvae was negative (Fig. 2A, lanes 2, 3 and 4 versus 1). When standard hPTH(1–84) was loaded on the gel, it appeared as a 9.4-kDa form as expected (Fig. 2A, lane 6). However, when the same standard was mixed with the control-hemolymph, the same two different immunoreactive peptides appeared (9.4 kDa and 14.3 kDa) (Fig. 2A, lane 5). Thus, the 14.3-kDa band appeared to be a hPTH-binding protein as also confirmed in subsequent analysis. hPTH production increased during this period and the highest level was obtained after 72 h, whereafter the larvae succumbed to an infection. A semiquantitative estimation of hPTH in hemolymph collected the 3rd day of infection was carried out. Comparing the intensities of immunoreactivity to the different amounts of known hPTH standards as shown in Fig. 2B when different amounts of hemolymph sample was analyzed, it was estimated that 4 µl contained 0.25–0.5 µg hPTH (Fig. 2B, lanes 5, 6 and 7 versus lanes 2, 3 and 4). The non saturable binding properties of the 14.3-kDa band was verified by addition

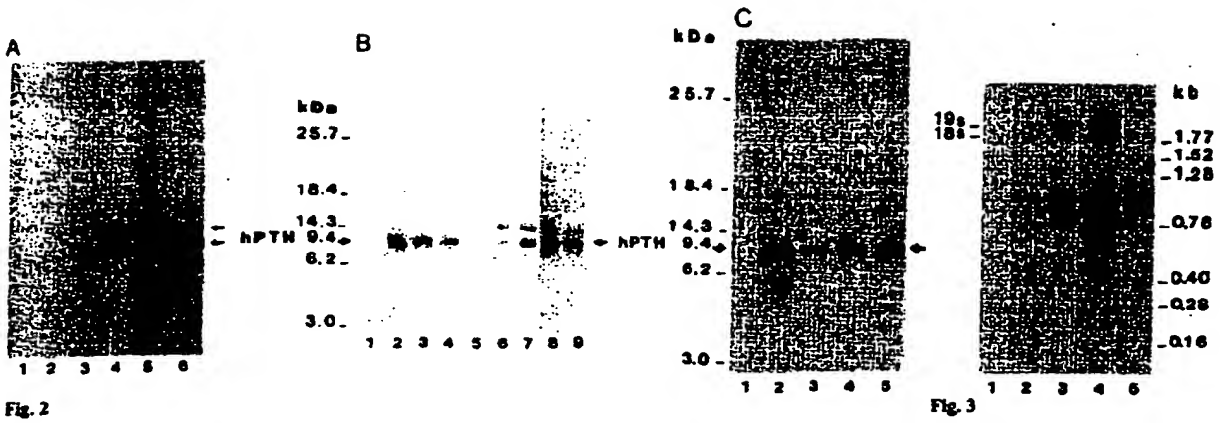


Fig. 2

Fig. 3

Fig. 2. The re-h*PTH* expression. (A) Time-course study of h*PTH* expression in hemolymph from infected larvae collected after 24, 48 and 72 h of infection with re-baculovirus (lanes 2, 3 and 4) compared to control represented by 72 h of wt infection (lane 1) using Western blot technique and mid-region specific anti-hPTH antibody. 3 µl per lane using 0.1% SDS-15% PAGE. Lane 5: hemolymph from wt infected larvae added 0.25 µg hPTH(1–84) standard. Lane 6: 0.25 µg hPTH(1–84) standard. (B) Semiquantitative estimation of hPTH produced in hemolymph 3 days after infection with wt (lane 1 (4 µl)) and re-baculovirus (lanes 5 (1 µl), 6 (2 µl) and 7 (4 µl)) and subjected to 0.1% SDS-15% PAGE, followed by immunoblot analysis. Lanes 2, 3 and 4: hPTH(1–84) marker (Bachem), 0.75 µg, 0.5 µg and 0.25 µg, respectively. Lanes 8 and 9: 4 µl of hemolymph from wt infected larvae added 0.5 µg and 0.25 µg hPTH(1–84) marker, respectively. (C) hPTH in BmN cell cultures 4 days after infection with re-virus (lane 2) and wt virus (lane 1). 5 ml of culture medium partly purified on a Sep-Pak column freeze-dried and analyzed by SDS–PAGE as in panel B. Lanes 3, 4 and 5: 0.05 µg, 0.1 µg and 0.075 µg, respectively, of hPTH(1–84) standard. **Methods**: *Bm larva and BmN cell culture*: The silkworm *Bm* larvae (TW × NB4D2) were fed ad libitum on fresh mulberry leaves and reared in the laboratory following the method of Krishnaswamy et al. (1973). BmN cells were grown in TC-100 medium containing 10% fetal calf serum and 50 µg gentamycin per ml at 27°C (Maeda, 1989a,b). *Transfection and isolation of re-virus*: Re-vector pBmPTH84 was amplified and purified. Subconfluent monolayers of BmN cells were co-transfected with purified infectious *Bm* wt baculovirus (BmNPV) DNA and the re-vector pBmPTH84. Homologous recombination between the plasmid DNA and wt viral DNA occurred in the Ca-phosphate mediated cotransfected cells as tested after 5 days by plaque assay, and the polyhedrin-negative plaques were screened for hPTH production in the BmN cells and *Bm* larvae. *Collection of larval hemolymph, fatbody, BmN cell culture medium and cell lysate*: Early fifth instar (24 h old) *Bm* larvae were needle inoculated with 50 µl of recombinant viral solution ($3 \times 10^5$ pfu) into the body cavity using wt virus and saline injections as controls. Hemolymph was collected and treated as described (Maeda, 1989a). BmN cells ($7.5 \times 10^6$ cells) were seeded in a tissue culture flask and after overnight incubation, the cells were infected by re-virus or wt virus (10 pfu/cell) separately. After 4 days of infection the medium was collected, the samples centrifuged at 1400 rpm for 5 min and the medium and cell pellet stored separately. All the samples were stored at −70°C till further analysis. *Protein determination*: Protein in hemolymph was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. *PAGE and immunoblotting*: 0.1% SDS-15% PAGE was performed under reducing conditions (Laemmli, 1970) and samples were solubilized as previously described (Towbin et al., 1979; Gabrielsen et al., 1990). *Semiquantitative protein determination of hPTH*: Semiquantitative measurements of hPTH using light scanning were performed on X-ray films developed after Western immunoblots using Bio Image system, Millipore.

Fig. 3. mRNA was prepared from fatbody cells and analyzed on agarose gels followed by Northern blot and hybridization to a h*PTH* cDNA fragment. Lanes: 1, 1.3 µg mRNA from wt-infected larvae (control) 72 h post-infection; 2, 3 and 4, 1.3 µg mRNA from re-virus-infected larvae 24, 48 and 72 h after infection; 5, 1 µg of PTH-mRNA isolated from human parathyroid adenomas. Ribosomal RNAs corresponding to 18S and 19S, respectively, are indicated. **Methods**: Total RNA was extracted from larval fatbody (wt virus infected and re-virus infected) as described (Glisin et al., 1974). Poly(A)$^+$RNA was selected from identical amounts of total RNA from all the extractions using magnetic oligo(dT) Dynabeads (Dynal A.S. Norway). For time-course response, RNA was extracted at every 24 h post infection for a period of 3 days. The poly(A)$^+$RNA samples were subjected to electrophoresis on a 1.5% agarose gel containing 6% (v/v) formaldehyde in 20 mM Na-phosphate pH 7.0 buffer. The RNA was subsequently transferred to a nylon membrane by passive diffusion and immobilized by UV light (2 min) and baked at 80°C for 1 h. The h*PTH* cDNA *Xba*I-*Eco*RI fragment was used for probe, and filters were subjected for hybridization at 42°C following standard procedures (Sambrook et al., 1989).

of 0.5 µg and 0.25 µg of hPTH, respectively, to the wt hemolymph (Fig. 2B, lanes 8 and 9) which by itself contained non-detectable immunoreactivity (Fig. 2B, lane 1).

**(c) Production and secretion of hPTH by BmN cells into culture medium**

BmN cells infected with re-virus also produced and secreted hPTH into the medium. Two secreted proteins were detected on immunoblots of SDS-PAGE using mid-region specific anti-h*PTH* antiserum (Fig. 2C, lane 2). One comigrated with hPTH(1–84) standard (9.4 kDa) and the other and somewhat diffuse 5.5-kDa immunoreactive band probably represented proteolytic degradation products. The amount of hPTH was estimated to be about 0.3–0.5 µg from the flask with an initial concentration of $7.5 \times 10^6$ cells as determined by light scanning of the X-ray films developed after various times and compared to hPTH standards (lanes 3, 4 and 5). No immuno-

reactivity was found in medium from wt virus-infected cells (Fig. 2C, lane 1).

**(d) The presence of h*PTH* mRNA**

The presence of h*PTH* mRNA in fatbody cells was also studied 24, 48 and 72 h after infection and analyzed on agarose gels followed by Northern-blot and hybridization to a *PTH*cDNA *Xba*I-*Eco*RI fragment (Fig. 1) as probe. A time-dependent increase (about 100-fold) in transcripts corresponding in size to *PTH*mRNA prepared from human parathyroid adenomas was observed (Fig. 3, lanes 2, 3 and 4 versus lane 5). In addition, two higher $M_r$ transcripts appeared of sizes equal to 18S and 19S rRNAs.

The presence of three mRNA species in the fat body may indicate heterogeneity within the non-translated regions since only one peptide form was demonstrated. They cannot be due to non-specific hybridization to remaining ribosomal RNA, since wt RNA gave no signal (Fig. 3, lane 1); in addition, a time-dependent increase was also observed.

**(e) Intracellular hPTH in larvae and in cultured cells**

Intracellular proteins from larval fatbody and BmN cells infected with re-virus were examined using immunoblots and compared to wt virus-infected controls. No hPTH immunoreactivity was detected in the larval fatbody cells while BmN cells showed two dominant hPTH immunoreactive bands. The major one of 13.5 kDa was similar to unprocessed prepro-hPTH while the 16-kDa protein could represent a modified variant or protein bound form of prepro-hPTH (data not shown).

**(f) Quantitative measurements of hPTH by two-site chemiluminometric (sandwich) immunoassay**

hPTH(1–84) in the hemolymph and culture medium was assayed using chemiluminometric immunoassay according to the manufacturer (Magic Lite, Ciba Corning, Germany). In hemolymph collected three days after coelomic infection, the hPTH concentrations were 0.05–0.1 g/l, while the total protein concentration was 63 g/l. In BmN cell culture medium at day 4 postinfection, the maximal hPTH concentrations were 40–70 μg/l per $10^6$ cells.

**(g) Reverse-phase HPLC-purification of hPTH and assessment of the chemical purity and authenticity**

The re-hPTH was extracted from hemolymph and further purified as described in Methods to Fig. 4. The HPLC purification profiles are shown in Fig. 4 (A, B and C). hPTH from the last HPLC-step (Fig. 4C) was analyzed further on SDS-PAGE (Fig. 4D and E). The results from the gel analyses, including silver-staining (Fig. 4D) and immunoblot analysis (Fig. 4E) showed only one band with a mobility equal to standard hPTH and a purity equal to or better than the hPTH(1–84) Bachem standard (Fig. 4D and 4E, lanes 1 and 3 compared to lanes 2 and 4).

The purified PTH was also subjected to aa composition analysis and N-terminal sequencing which were consistent with the theoretical prediction (data not shown). Mass spectrometry was performed with a spectrum as shown in Fig. 5A and an $M_r$ of 9425 was obtained from the single-charged molecular ions corresponding well

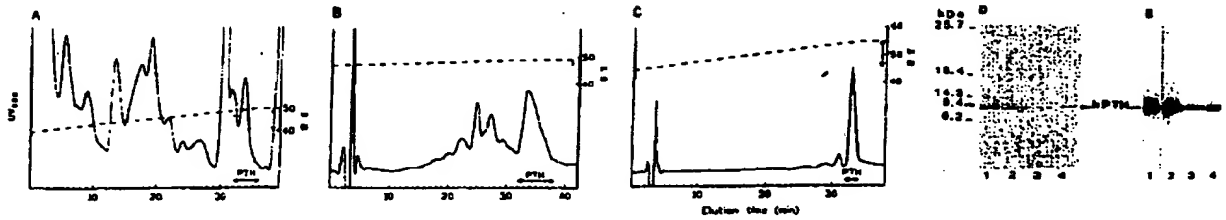


Fig. 4. Separation of re-hPTH(1–84) by reverse-phase HPLC from hemolymph of infected silkworms (A, B and C) and characterization by SDS-PAGE (D and E). (A) Preparative scale chromatography was performed on a Pharmacia SuperPac Pep-S $C_{18}/C_2$ column (22.5 × 250 mm) as previously described by Olstad et al. (1992) with small modifications. Eluant A, 0.115% TFA in MilliQ water; eluant B, 0.085% TFA in 70% aqueous acetonitrile (ACN) (10 ml/min). (B) Pooled fractions from A) containing hPTH were analyzed on an analytic Pharmacia SuperPac Pep-S $C_{18}/C_2$ column (4.0 × 250 mm) (linear gradient of 47–49% eluant B run for 35 min). Eluant A and eluant B were the same as in A. The flow rate was 1.0 ml/min. (C) Pooled fractions from B) containing hPTH were further purified on Pharmacia SuperPac Pep-S $C_{18}/C_2$ column (4.0 × 250 mm). A linear gradient of 45–55% eluant B was run for 35 min. Eluant A, 0.55% TFA in MilliQ water; eluant B, 0.45% TFA in 70% aqueous ACN. The flow rate was 1.0 ml/min. (D and E) 0.1% SDS-15% PAGE analysis of HPLC purified hPTH from hemolymph (Fig. 3C, fractions 33 and 34) and compared to hPTH(1–84) standard from Bachem. (D) Silver-staining. (E) Immunoblot analysis using the mid-region specific anti-hPTH antiserum. Lanes 1 and 3: 1.0 μg and 0.2 μg of hPTH from hemolymph. Lanes 2 and 4: 1.0 μg and 0.2 μg hPTH(1–84) standard (Bachem). Methods: *Purification of hPTH from the medium*: hPTH was concentrated and partly purified as described previously (Olstad et al. 1992) and after freeze-drying, the samples were dissolved in sample-buffer for SDS-PAGE analyses (Laemmli, 1970). *Reverse-phase HPLC*: Preparative and analytical scale chromatography was performed as described previously on SuperPac Pep-S $C_{18}/C_2$ column (22.5 × 250 mm/4.0 × 250 mm) (Reppe et al., 1991; Olstad et al., 1992). For silver staining of the gel, the procedure for the Sigma silver stain kit was followed.

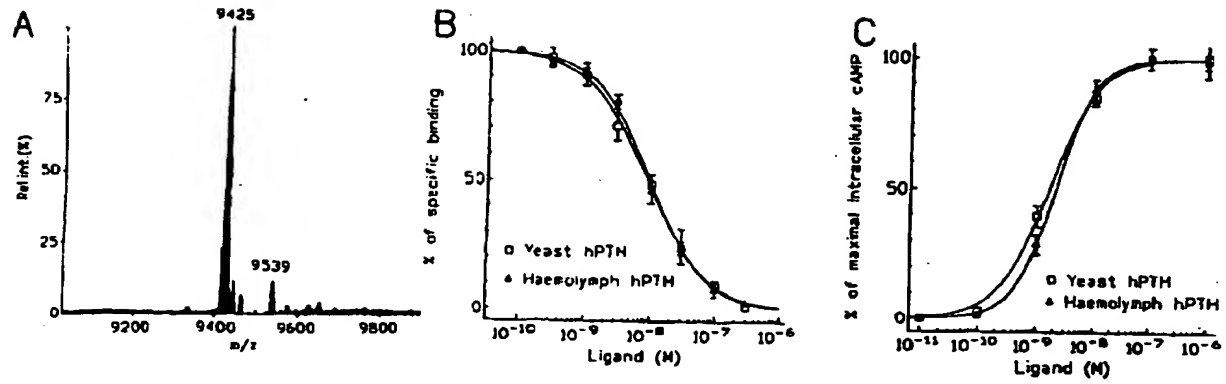


Fig. 5. Characterization of re-hPTH by mass-spectroscopy (A), radioreceptor binding (B) and intracellular cAMP stimulation (C). (A) Mass-spectroscopic analysis of recombinant hPTH(1–84). The dominant single peak represents a molecular hPTH mass of 9425 Da corresponding to the theoretical value of 9424.7 Da. (B) Inhibition of radiolabeled [Tyr$^{36}$]chicken-PTH-related protein(1–36)amide by different hPTHs. The re-hPTH(1–84) produced in infected silkworms and recombinant hPTH(1–84) (yeast hPTH) produced in *Saccharomyces cerevisiae* were tested in a radioreceptor assay using LLC-PK$_1$ cells transfected with the rat PTH/PTHrP receptor. The data represent the mean ± SEM of three independent experiments each performed in triplicate. (C) Stimulation of cellular cAMP by different hPTHs. Accumulation of intracellular cAMP in LLC-PK$_1$ cells transfected with the rat PTH/PTHrP receptor and stimulated (15 min, 37°C) with re-hPTH(1–84) produced in silkworms and re-hPTH(1–84) (yeast hPTH) produced in *Saccharomyces cerevisiae* is shown. The data represent the mean ± SEM of three independent experiments each performed in duplicate. Methods: *Mass spectrometry analysis* was performed using a API III LC/MS/MS system (Sciex, Thornhill, Ontario, Canada) and carried out as described (Covey et al., 1988). *Radioreceptor assay*: LLC-PK$_1$ cells expressing the rat PTH/PTHrP receptor (Bringhurst et al., 1993), were plated in 24-well plates (50 000 cells/well) and grown for 2 days before incubation with $^{125}$I-labelled [Tyr$^{36}$]chicken-PTHrP(1–36)NH$_2$ (100 000 cpm per well/0.5 ml) in the presence or absence of competing ligands at 15°C for 4 h, using a Tris-based binding buffer (50 mM Tris-HCl, pH 7.7/100 mM NaCl/5 mM KCl/2 mM $CaCl_2$/5% heat-inactivated horse serum/0.5% heat-inactivated fetal calf serum) as described (Jüppner et al., 1988). The competing ligands were recombinant hPTH(1–84) expressed in yeast (yeast hPTH) (Gabrielsen et al., 1990; Olstad et al., 1992) and recombinant hPTH(1–84) purified from hemolymph of infected silkworms. Techniques used for radioiodination of PTHrP analog were previously reported. *Intracellular cAMP measurements*. Measurements of intracellular cAMP in LLC-PK$_1$ cells expressing the rat PTH/PTHrP receptor (Bringhurst et al., 1993) using Dulbecco's modified Eagle's medium containing 2 mM 3-isobutyl-1-methylxanthine and 0.1% bovine serum albumin. Medium (0.5 ml) with or without PTH was added and cells were transferred to a 37°C water bath for incubation in 15 min, then washed and immediately frozen on liquid nitrogen. Intracellular cAMP was measured by a radioimmunoassay kit from Amersham, after lysing the cells with 1 ml of 0.05 M HCl. The stimulating ligands were re-hPTH(1–84) produced in yeast (yeast hPTH) (Gabrielsen et al., 1990; Olstad et al., 1992) and re-hPTH(1–84) produced in *Bm* hemolymph.

with the theoretical $M_r$ of 9424.7 for hPTH as calculated from the aa composition.

**(h) Radioreceptor binding studies and intracellular cAMP measurements**

Binding properties of two different re-hPTH forms from yeast and silkworm are shown in terms of displacement curves using the $^{125}$I-labeled [Tyr$^{36}$]chicken-PTHrP(1–36)NH$_2$ as radioligand and LLC-PK$_1$ cells permanently transfected with the rat PTH/PTHrP receptor. Both hPTH forms representing the authentic hormones, showed equal receptor binding affinities (Fig. 5B) (calculated $K_d = 8.8 \pm 1.2 \times 10^{-9}$ M) and identical abilities to stimulate intracellular cAMP accumulation in the same cells with half maximal response obtained at $2.0 \times 10^{-9}$ M (Fig. 5C).

**(i) Concluding remarks and comparison of h*PTH* expression between various host systems**

The quantitative result of re-hPTH production in BmN ovarian cell culture was much less than that in the hemolymph even corrected for the 30%–50% loss during medium concentration and Sep-Pak column chromatography (see Methods in the legend to Fig. 4). The circulatory system of silkworm larvae opens into the coelomic cavity which is totally bathed in the hemolymph and retains the secretory proteins (Shigematsu, 1958).

hPTH could not be detected in the intracellular fractions of larval fatbody while the polypeptide and the assumed unprocessed forms were present in BmN cells. Ovarian cells are normally not designed for secretion of proteins, but rather for absorption, and this may be a reason for the low level of hPTH produced. The hormone and its mRNA were expressed in a parallel and a time-dependent fashion. The hPTH produced in the silkworm larvae was authentic as judged by N-terminal sequence, total aa composition and mass spectrometry. Also its receptor binding affinity and ability to activate the main second messenger system were identical to the yeast re-hPTH which previously was shown to have full biological activity in several target cell systems (Reppe et al., 1991).

The amount of hormone produced in the larval hemolymph represented 70 mg/l. This level was many times

higher than that reported for yeast (Gabrielsen et al., 1990; Reppe et al., 1991) and for *E. coli* (Høgset et al., 1990) secreted hPTH. The re-hPTH produced as an intracellular fusion protein with *S. aureus* protein A, showed a production after purification of 50–80 mg/l culture (Forsberg et al., 1991).

The production yield of hPTH in the silkworm larvae also compared well to ZZ-cecropin A fusion protein production in *Trichoplusia ni* larvae using *Autographa californica* baculovirus (Andersons et al., 1991). Production of human α-interferon in silkworm using *Bm* baculovirus, amounted to 30 mg/l hemolymph after purifying 10 ml of hemolymph by affinity column chromatography (Maeda et al., 1985). However, they did not report the concentration of α-interferon in the hemolymph prior to purification.

Expression of h*PTH* in microbiological systems has met with two problems related to incorrect N-terminal cleavage and aberrant intracellular processing (Rabbani et al., 1988; Høgset et al., 1990; Gabrielsen et al., 1990; Reppe et al., 1991; Forsberg et al., 1991; Rokkones et al., 1994). The major cleavage sites were after $Lys^{26}$ in *S. cerevisiae* and after $Val^{21}$ in *E. coli* (Rokkones et al., 1994). In mammalian cells, in contrast (mouse mammary tumor cell, i.e., C1271 cells, and Chinese hamster lung cells, i.e., DON cells) the entire h*PTH* cDNA including the prepro part gave rise to only the intact form (Rokkones et al., 1994). We demonstrate that the silkworm larvae in fact resembles the mammalian system cleaving the hPTH signal sequence correctly and that the human signal is also able to promote an efficient secretion of the intact hormone. However, the hPTH produced binds to a natural protein in the hemolymph and gives rise to a 14.3-kDa protein in addition to the expected 9.4-kDa form. The N-terminal sequence of the purified '14.3-kDa protein' was identical to hPTH(1–84) (data not shown). Standard hPTH also showed the same two bands when added to the hemolymph (Fig. 2A and B). Moreover, in buffer containing urea, the mobility of the 14.3-kDa form was normalized (data not shown) and also the 'acid treatment' occurring during HPLC purification released the peptide. Thus, the '14.3-kDa protein band' represents a hPTH-binding protein of unknown nature.

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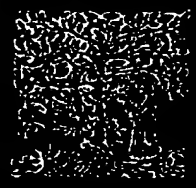

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